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Chapter

Regulation of Exosomes in the Pathogenesis of Breast Cancer

Congjian Shi, Hongqin Yang, Zhengchao Wang and Zhenghong Zhang

Abstract

Extracellular vesicles (EVs) are a heterogeneous group of endogenous nanoscale vesicles that are secreted by various cell types. Based on their biogenesis and size distribution, EVs can be broadly classified as exosomes and microvesicles. Exosomes are enveloped by lipid bilayers with a size of 30–150 nm in diameter, which contain diverse biomolecules, including lipids, proteins and nucleic acids. Exosomes transport their bioactive cargoes from original cells to recipient cells, thus play crucial roles in mediating intercellular communication. Breast cancer is the most common malignancy among women and remains a major health problem worldwide, diagnostic strategies and therapies aimed at breast cancer are still limited. Growing evidence shows that exosomes are involved in the pathogenesis of breast cancer, including tumorigenesis, invasion and metastasis. Here, we provide a straightforward overview of exosomes and highlight the role of exosomes in the pathogenesis of breast cancer, moreover, we discuss the potential application of exosomes as biomarkers and therapeutic tools in breast cancer diagnostics and therapeutics.

Keywords: extracellular vesicles, exosomes, breast cancer, miRNAs

1. Introduction

Extracellular vesicles (EVs) are heterogeneous membrane-bound vesicles which originate from endosomal or plasma membrane called exosomes or microvesicles, respectively [1]. The release of EVs was initially identified as a mode for cells to eliminate unwanted substances, however, the initial view with regard to EVs has changed dramatically with the deepening of research, and their crucial roles in diverse physiological and pathological processes have attracted extensive attention. According to their original cells, EVs are loaded with a specific set of preassembled bioactive cargoes, and give rise to phenotypic and genotypic changes in recipient cells [2, 3]. These cargoes enclosed within EVs are biologically significant, for example, three EV subtypes including one microvesicle and two exosome populations released by LIM1863 CRC (colorectal cancer) cells have distinct miRNA expression profiles [4]. EVs contribute to numerous aspects of normal physiological processes, including blood coagulation, immune surveillance, tissue repair and stem cell maintenance [5]. They are also closely related with diverse human diseases, including cancer, infectious diseases, neurologic diseases and cardiometabolic diseases [6]. Exosomes are a subtype of EVs and the application of exosomes as biomarkers

and therapeutic tools has appeared as a promising area of research due to some preponderant properties of exosomes. Exosomes can be released according to the command received from adjacent and distant cells, or in response to the stimulation induced by local conditions [7]. Both normal and pathological cells are capable of secreting exosomes and they are stable in biological fluids [8]. Breast cancer is the most common malignancy affecting women, and its morbidity and mortality are estimated to increase in the coming years [9]. One in eight to ten women will be diagnosed with breast cancer during their lifetime [10], and breast cancer has seriously affected women's health. Accumulating evidence indicates that exosomes are involved in the pathogenesis of breast cancer, including tumorigenesis, invasion and metastasis. Studies focused on exosomes might provide novel perspectives for revealing breast cancer pathogenesis and improving the current poor diagnostic and therapeutic status of breast cancer.

2. Extracellular vesicles

Cells naturally release EVs into the extracellular space, these nanoscale vesicles encompassing bioactive cargoes play crucial roles in diverse physiological and pathological processes. The term EVs represent several subtypes of vesicles, standardized criteria for distinguishing EVs subtypes are still under discussion, but it is universally acknowledged that they can be classified as two main categories: exosomes and microvesicles. Other EVs subtypes such as apoptotic bodies [11], speresomes [12] and large oncosomes [13], are not mentioned in this review. Exosomes have endosomal origin, they are 30–150 nm in diameter and float at a density of 1.13–1.19 g/ml in sucrose gradient [14, 15]. Exosomes are essentially intraluminal vesicles (ILVs) generated by inward budding of endosomal membrane during the maturation of endosomes, then released to the extracellular space when multivesicular bodies (MVBs) (also referred to late endosomes) fuse with plasma membranes [16, 17]. Microvesicles, typically larger than exosomes (100–1000 nm in diameter), arise through direct outward budding and fission of plasma membrane [18], hence, the membrane composition of microvesicles can better reflect the membrane composition of original cells in contrast to exosomes. Although the origin of exosomes and microvesicles occurs at distinct intracellular locations, some common mechanisms participate in both processes. The modes by which recipient cells take up EVs including endocytosis, direct membrane fusion and receptor ligand binding [19], but the specific molecular mechanisms deserve further investigation.

At present, the biogenesis of EVs, the substances they contain and the biological effects they promote have been extensively studied, which make people find out the potential of EVs in clinical application. EVs subtypes like exosomes and microvesicles may perform different functions, and it is absolutely necessary to isolate high-purity EVs subtypes, which will be crucial for EV-related functionality and therapeutic value studies. But even in EV preparations with high-purity, electron microscopy (EM) results imply that they still contain co-purifying elements [20]. The isolation of EVs is challenging because EVs subtypes have some similarities, including their size, density, composition, and surface marker proteins [21]. Meanwhile, EVs derived from biological fluids contain a mixture of multiple EVs secreted by various cell types [22]. Therefore, it is imperative to formulate universal standard protocols for the preparation of EVs.

Due to some peculiar characteristics of EVs, they have prominent biotechnological potential. EVs are biocompatible and safe, coupled with nanoscale diameter, resulting in their long blood circulation half-life and high drug loading capacity, which makes them possible to be ideal drug delivery vehicles [23]. EVs represent

an attractive group of therapeutic biomarkers and has tremendous potential in immune response regulation and tissue regeneration [5, 24]. EVs are extensively found in diverse bodily fluids, and it is a promising area to serve EVs as biomarkers for early diagnosis and accurate prognosis. Since EVs are derived from bodily fluids, the diagnostic methods are probably non-invasive and considerably less painful than some existing diagnostic methods (for example, liver biopsy). Meanwhile, the clinical application of EVs can also monitor the response of therapy, which will contribute to convalescent process.

3. Exosomes

Exosomes are enveloped by lipid bilayers and act as mediators of intercellular communication through transmitting diverse functional biomolecules from original cells to recipient cells, and they are secreted by virtually all cell types, such as stem cells, immune cells and tumor cells [25]. The cargoes transported by exosomes including lipids, proteins, RNA (coding and non-coding) and even DNA (genomic and mitochondrial) [26]. Exosomes can be detected and isolated from diverse bodily fluids, exemplified by blood, urine, saliva, cerebrospinal fluid and breast milk [27], they can also be obtained from cell culture-conditioned media [28]. Some specific surface proteins are considered as the makers of exosome, such as tetraspanin family (CD9, CD63 and CD81), heat shock protein 70 (HSP70) and major histocompatibility complex (MHC) molecules [29]. Exosomes also contain abundant ceramide, cholesterol and sphingomyelin, which may relate to their lipid raft microdomains [30]. Multiple genetic materials are detected in exosomes, and exosome-encapsulated miRNAs have obtained extra attention because of their vital roles in regulating gene expression and can be used as biomarkers for a variety of diseases [31].

Exosomes exhibit unique biogenesis mechanism. Plasma membrane buds inward through endocytosis, resulting the generation of early endosomes [32]. The process from early endosomes to late endosomes (also referred to MVBs) requires the involvement of Golgi complex, during which ILVs also accumulate by the invagination of endosomal membrane in their lumen [15]. Then, MVBs either fuse with lysosomes, which ILVs are degraded, or fuse with plasma membranes, which ILVs are released to the extracellular space as exosomes [33]. Fusion of MVBs with plasma membrane requires the assistance of soluble N-ethylmaleimide-sensitive fusion attachment protein receptor (SNARE) complexes [34]. The endosomal sorting complex required for transport (ESCRT) machinery, a vital participant in exosome biogenesis, is responsible for ILVs formation and protein sorting [35]. ESCRT machinery contains four complexes, ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III, as well as associated proteins, including ALIX, VPS4 and VTA1 [36]. ESCRT-0 recognizes the ubiquitinated cargoes, ESCRT-I and ESCRT-II initiate the budding process of ILVs, whereas ESCRT-III terminates this process [3, 37]. ESCRT-independent mechanisms also exist by the evidence that MVBs still form in the absence of ESCRTs [38], further studies report that the mechanisms are related with the sphingolipid ceramide [39] or some members of the tetraspanin family [40]. In single MVBs, a competitive relationship between ESCRT-dependent and ESCRT-independent mechanisms exists, which affects the size of ILVs formed inside [41], this makes it possible to identify different subpopulations of MVBs based on their ILVs size. A group of Rab GTPases including Rab11, Rab27a, and Rab27b, are also involved in the release of exosomes [42]. Once exosomes are released to the extracellular space, they may exist in the circulation or be taken up by adjacent and distant cells [43, 44].

Exosomal preparations with high-purity are significant for further exploration of exosomal biogenesis and functions, and techniques for exosomal isolation have made great advances. At present, the commonly used isolation techniques including ultracentrifugation-based techniques, size-based techniques, precipitation, immunocapture and microfluidic-based techniques [35, 45] (**Table 1**). Among them, ultracentrifugation is the most extensively used exosomal isolation technique for bodily fluids and cell culture supernatant [46]. Each technique has its own merits and demerits, and the combination of aforementioned techniques may lead to a more desirable isolation. Recent study showed that the acidic condition was more suitable for the isolation of exosomes [47], indicating that local pH of exosomes should be taken into account for future researches.

Specific roles of the tumor microenvironment during cancer progression and metastasis have been widely studied [48], and cancer cell–derived exosomes can establish a favorable microenvironment to induce cell proliferation, angiogenesis, resistance to apoptosis and initiation of pre-metastatic niches through their bioactive content [22, 49]. The secretion of exosome appears to have an impact on drug resistance, for example, exosomes enriched in TAG72 imply that CRC patients might be resistant to 5-FU [50]. And cells under pathological status release even more exosomes, it is estimated that there are approximately 2,000 trillion exosomes presented in normal human blood and 4,000 trillion exosomes presented in the blood of cancer patients [51]. According to these results, it is feasible to serve exosomes as biomarkers for diagnosis and prognosis. Exosomes are capable of inducing anti-tumor responses through delivering tumor antigens to immune cells, and exosomes derived from T cells can suppress tumor development [52], demonstrating their great potential in modulating immune responses. Enlightened by the capability of exosomes that transmits biomolecules from original cells to recipient cells, accompanied with their biocompatibility, low immunogenicity and toxicity, high stability in the circulation, biological barrier permeability and potential targeting to specific sites [53], diverse strategies have been developed for loading therapeutic cargoes into exosomes, which have a broad application prospect.

Exosome plays important roles in tumor diagnostics and therapeutics. Tissue biopsy is usually acquired from the site of primary tumor and reflects its molecular traits over a period of time, therapies will be determined according to the results of tissue biopsy. However, the limitations of tissue biopsy are obvious, it is not comprehensive enough to reflect heterogeneity and dynamic evolution of tumor [54].

Isolation techniques	Advantages	Disadvantages	References
Ultracentrifugation-based techniques	Low cost, most commonly used	Time-consuming, high equipment cost, low recovery	[15, 45]
Size-based techniques	Fast, convenient, high yield	Lack specificity, requires dedicated equipment	[17, 46]
Precipitation	Easy, does not require specialized equipment	Lack specificity, time-consuming	[15, 45, 46]
Immunocapture	High purity	Expensive, low capacity	[28, 35]
Microfluidic-based techniques	Fast, low cost, convenient	Lack standardization and large-scale tests on clinical samples, lack method validation	[45, 52]

Table 1.
The advantages and disadvantages of different techniques used for exosome isolation.

Exosome related liquid biopsy techniques including surface-enhanced Raman spectroscopy (SERS), next generation sequencing (NGS), digital droplet PCR (ddPCR) and molecular barcoding, have drawn extra attention due to its unique advantages of minimally invasive and serial biochemical tests [55]. Among diversified methods developed for liquid biopsy, SERS-based technique for detection of circulating tumor markers including exosomes is one of the most powerful methods, it owns the advantages of high sensitivity, specificity, tremendous spectral multiplexing capacity for simultaneous target detection, and its unique capability for obtaining intrinsic fingerprint spectra of biomolecules [56]. The application of exosome related liquid biopsy enables the improvement of various aspects of tumor management including early diagnosis and screening, prediction of prognosis, early detection of relapse, serial sampling and efficient longitudinal monitoring of disease progress and response to treatment [57]. Although exosome related liquid biopsy is a promising area, there are still some loopholes including difficult extraction and did not analyze the phenotypic studies of cells from tumor, that require further refinement and validation [58].

4. Breast cancer

Breast cancer is a disease with high heterogeneity, containing multiple tumor entities that have diverse clinical behavior and biological features [59], which complicate its diagnosis and treatment. Among women, breast cancer is the most common malignancy and the second leading causes of cancer-related death [60]. The 5-year overall survival rate for non-metastatic breast cancer patients is greater than 80%, whereas distant metastasis can reduce this rate to approximately 25%, and the main metastatic sites including bone, brain, liver and lung [61]. The diagnosis and treatment of patients are evaluated by clinical assessment, breast imaging, tumor size, histologic grade, lymph node involvement or acknowledged biomarkers, including estrogen receptor (ER), human epidermal growth factor receptor 2 (HER2) and progesterone receptor (PR) [62]. The clinical classification of breast cancer should be reasonable, so as to select the most appropriate diagnostic strategy and therapy for each patient, and the molecular subtypes of breast cancer are represented by basal-like, HER2-enriched, normal breast-like, luminal A, luminal B and claudin-low [63]. The occurrence of breast cancer is influenced by age, race, obesity, smoke, drinking, oral contraceptives and other exogenous estrogens, age at menarche, age at menopause, age at first live birth and environmental toxins [64, 65], also, inherited genetic mutations are responsible for 5–10% of all breast cancer cases, and mutations in BRCA1 and BRCA2 are believed to increase the lifetime risk of being diagnosed with breast cancer by more than four times [66].

Breast cancer is generally diagnosed by mammography, computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, core needle biopsy, excisional biopsy and histopathologic evaluation [65, 67]. Diagnosed patients with parallel clinical and biological characteristics may exhibit distinct responses to treatment and bring about different outcomes [68], therefore, the research on breast cancer treatment needs to be further deepened. At present, surgery and radiation are mentioned frequently in treatment, and three approaches are primarily adopted in medical oncology: ER + -related breast cancer aimed at anti-endocrine strategies, HER2 + -related breast cancer treated with HER2-targeted drugs and triple-negative breast cancer (TNBC) managed with traditional cytotoxic therapy [69]. More importantly, it is not just about choosing the appropriate treatment for each patient, the sequencing of therapies should also be considered.

5. Exosomal functionality and therapeutic value in breast cancer

It has been widely acknowledged that exosomes are important players in the pathogenesis of breast cancer (**Figure 1**). The release of exosomes induced by heparanase, hypoxia and other stimulation is involved in breast cancer angiogenesis, which facilitate tumorigenesis process of breast cancer [70]. Also, exosomes promote breast cancer tumorigenesis by modifying tumor microenvironment to permissive niches. Typically, alteration in miRNAs expression have been found to influence initiation and development of breast cancer [71], for example, compared with non-malignant breast cells or non-metastatic breast cancer cells, exosomal miR-10b is significantly upregulated in metastatic breast cancer cells [72]. Further research shows that RNA induced silencing complex-loading complex (RLC) proteins Dicer, AGO2 and TRBP, which have been proved to participate in miRNA biogenesis, can be detected in exosomes derived from the serum of breast cancer patients and breast cancer cells, moreover, Dicer inhibition in cancer exosomes obviously decelerates tumor growth in recipient cells [73]. Invasion plays an important role in cancer development, invasion ability of non-malignant breast cells can be induced by exosomes derived from metastatic breast cancer cells [72]. Metastatic breast cancer cells specifically express and release miR-105, during which exosomal miR-105 can transfer to endothelial cells and acts as an effective regulator of their migration [74]. Recent study also suggested that miR-7641 was identified as an important component of exosomes that could promote breast tumor metastasis [75]. Drug resistance are also closely related with exosomes as they are capable of transporting anti-cancer drugs outside breast cancer cells. Chen et al. reported that drug-resistant breast cancer cells might spread their drug-resistant capacity to sensitive cells through secreting exosomes, they further confirmed that this

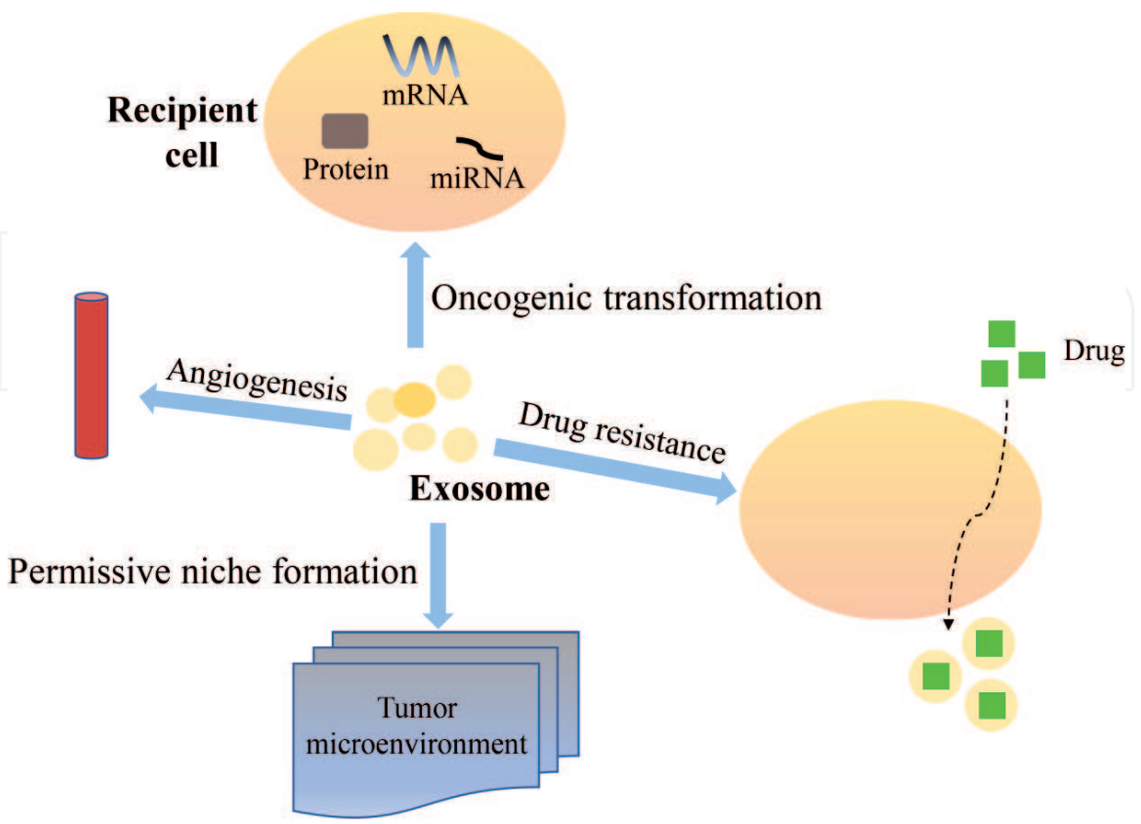

Figure 1.
Exosome in the pathogenesis of breast cancer. Exosome contribute to oncogenic transformation, angiogenesis, permissive niche formation and drug resistance in breast cancer.

process was mediated by exosomal miRNAs [76]. Trastuzumab is a commonly used drugs to treat breast cancer, while exosome-transmitted cicHIPK3 could promote trastuzumab chemoresistance of drug-sensitive BC cells, decreasing the therapeutic effect [77]. Recent study showed that exosomal miR-155 regulated drug resistance of breast cancer [78] and chemotherapy with miR-155-targeting therapies may lead to satisfactory outcomes.

Breast cancer is a disease which tends to metastasis, patients with early diagnosis, reasonable prognosis and accurate treatment usually have more favorable outcomes, yet approaches against breast cancer are still limited, and exosomes could be employed as novel biomarkers and therapeutic tools for patients with breast cancer. Hannafon and colleagues found that exosomes derived from breast cancer cells were enriched with specific miRNAs (miR-1246 and miR-21), what's more, these miRNAs in plasma exosomes of breast cancer patients were significantly higher than those of healthy control subjects [79], and exosomes may play crucial roles as biomarkers for breast cancer. Distant metastasis or local recurrence of breast cancer are strongly related with exosomal miRNAs, including miR-17-5p, miR-93-5p, miR-130a-3p, miR-340-5p [80], which can serve as indicators for prognosis. Now that exosomes remain stable in biological fluids, they are also promising for early diagnosis or monitoring the treatment process of breast cancer. In contrast to delivering anticancer drugs outside breast cancer cells, exosomes can also target anticancer drugs to breast cancer cells after appropriate modifications, for example, exosomes modified by targeting ligands deliver doxorubicin to tumors [81], which improve the therapeutic efficacy. Exosomes derived from mesenchymal stem cells (MSCs) can be used as drug delivery vehicles to transport locked nucleic acid (LNA)-anti-miR-142-3p, therefore reducing tumorigenicity in breast cancer [82].

6. Summary

Over the past few decades, on account of great advances in our understanding of breast cancer biology, diverse diagnostic and prognostic strategies, as well as targeted therapies are continuously evolving, while the situation of breast cancer patients remains unsatisfactory. For prevention and treatment of breast cancer, we need not only to develop new biomarkers and therapeutic tools, but also to further investigate the potential molecular mechanisms. Fortunately, accompany by our comprehension of exosomes is becoming more refined, the role of exosomes in initiation and development of breast cancer has been widely explored, and it is meaningful to translate exosomal research achievements to develop safe and effective therapies, diagnostic methods, along with drug delivery vehicles, which may conduce to improve the unsatisfactory situation of breast cancer patients.

Acknowledgements

This study was supported by the Special Funds of the Central Government Guiding Local Science and Technology Development (2020L3008), Provincial Natural Science Foundation (2018J01721, 2018J01721, 2019J01674 and 2020J01176), the Educational Reform Project (Y201809 and I202003009) and Training Program of Innovation and Entrepreneurship for Undergraduates (CXXL2020291 and CXXL2020293) of the Fujian Normal University.

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